



AMTicide[®] VAF

Code Number: FSSM14004
INCI Name: *Bacillus* Ferment & *Saccharomyces* Ferment Filtrate
INCI Status: Approved
REACH Status: Fully Compliant
CAS Number: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS Number: 295-779-9 (or) N/A & 232-387-9
Origin: Biotechnology
Processing:
GMO Free
No Ethoxylation | No Irradiation | No Sulphonation
No Ethylene Oxide treatment | No Hydrogenation
Additives: None | **Preservatives:** None | **Antioxidants:** None
Other additives: None
Solvents used: Propanediol
Appearance: Clear to Slightly Hazy Liquid
Soluble/Miscible: Water
Suggested Use Levels: 1.0% maximum
Suggested Applications: Skin Conditioning, Antifungal



BACKGROUND

Consumer demand and ever-changing regulations has driven the market from synthetic material to focus on natural solutions. Formulator Sample Shop[®] prides itself in supplying effective, natural products that provide skin and hair conditioning benefits, along with providing natural antimicrobial activity. **AMTicide[®] VAF** is developed by co-fermenting *Bacillus subtilis* with *Saccharomyces boulardii* in a defined growth media to deliver a non-irritating, effective, multifunctional product. This highly marketable product can provide antioxidant benefits and is capable of preventing the growth of fungus in packaging headspace, making it the perfect addition to any formulation.

SCIENCE

A gap in the market has been identified for a naturally derived volatile antifungal to prevent microbial growth associated with packaging. Packaging is a critical component for the successful preservation of cosmetic and personal care products. For a variety of packaging options, including jars, ensuring that the packaging headspace remains free of contamination is essential to prevent contamination of the entire formulation. Packaging processes are able to create a favorable growth environment for yeast and mold.

For example, leaving space between the contents of the packaging and the lid of the packaging allows sufficient room for microbial growth after a hot poured formulation has cooled. In addition, each time the packaging container is opened and the product is used, the headspace in the packaging increases, exposing more surface area of the formulation to the air. Phenoxyethanol, a synthetic volatile antimicrobial, has been commonly utilized to prevent headspace microbial growth. However, the potential for sensitization and sensory irritation of the skin associated with the use of phenoxyethanol¹, as well as stricter worldwide regulations on the material, pushes formulators to explore alternative options. A natural volatile antifungal solution has been successfully provided with the development of **AMTicide[®] VAF**.

AMTicide[®] VAF is a product of the co-fermentation of *Bacillus subtilis* and *Saccharomyces boulardii* in a defined growth medium. *Bacillus* spp. are well known rhizosphere residents of many crops, including tomato, corn, and soybeans, and produce nonvolatile and volatile secondary metabolites that exhibit antifungal activity as a mechanism of biocontrol to promote plant growth.^{2,3} The volatile organic metabolites produced by *Bacillus subtilis* have been known to naturally reduce and prevent plant diseases caused by fungi.

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Saccharomyces boulardii is a probiotic strain of yeast, first isolated from lychee and mangosteen fruit in 1934 by a French scientist by the name of Henri Boulard. *Saccharomyces boulardii* has sparked interest around the world, specifically due to its wide variety of interactions with other microbes and ability to disable gastrointestinal disorder and symptoms of gastrointestinal distress. Current research in the food industry has examined the ability of *Saccharomyces* spp. and lactic acid bacteria, such as *Bacillus* spp., to enhance the production of volatile compounds when co-inoculated.^{4,5}

The inoculation of *Saccharomyces boulardii* has been included in the fermentation process to enhance the bioactivity of the *Bacillus subtilis* volatile metabolites. Using bio-fermentation and various filtration techniques, the volatile organic metabolites are isolated and extracted from the bacteria cell to deliver high potency volatile antifungal activity. This method has successfully been able to produce a natural derived, high potency volatile material that delivers moisturizing and antifungal activity for effective headspace protection!

AMTicide[®] VAF was developed to be used in conjunction with one of our broad-spectrum antimicrobials, however it can be used alongside any preservative package for extra protection against yeast and mold associated with packaging.

BENEFITS

A skin moisturization study was performed using an untreated control, generic cream base, and the same cream base containing 1.0% **AMTicide[®] VAF**. Comparative moisturization results from this study are shown in Figure 1.

As demonstrated by the results of this study, the addition of 1.0% **AMTicide[®] VAF** improved moisture levels by 16.50% after 24 hours and by 34.0% after four weeks when compared to the untreated control. When compared to the base cream **AMTicide[®] VAF** improved moisturization by 3.96% and after 24 hours and by 9.88% after four weeks. Results indicate that **AMTicide[®] VAF** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

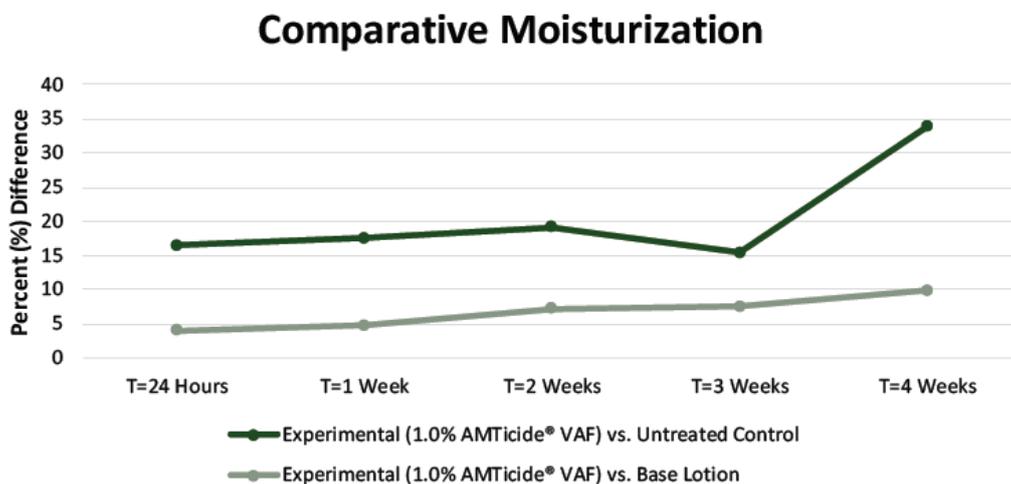


Figure 1. Comparative Moisturization.

A Headspace Preservation Test was then conducted over a period of 28 days to evaluate the preservation adequacy of 1.0% **AMTicide[®] VAF** compared to phenoxyethanol (positive control) and an unpreserved generic cream base (negative control). *Penicillium brevicompactum* and *Fusarium* sp. were isolated from the environment via passive sedimentation, to observe a 'real-life' example of contamination from manufacturing areas, storage conditions, or consumer use.

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The base cream formula used to perform the test was poured into individual cosmetic containers at 45°C in order to create the best environment for mold to grow under the test conditions. Each plastic jar was filled to approximately 85% of its capacity (~25ml) leaving 25% capacity of each jar empty as headspace. 1.0% **AMTicide**[®] **VAF** and 1.0% phenoxyethanol were subsequently added separately. Each cap of the cosmetic containers was inoculated separately with *Penicillium brevicompactum* and *Fusarium* sp. Each cap was tested at 7, 14, 21 and 28 days after the initial inoculation. Figure 2 represents the percent reduction of viable organisms after being introduced into the test formulation.

| Test Product | Testing Day | <i>Penicillium brevicompactum</i> 2.4 x 10 ⁶ CFU/ml | <i>Fusarium</i> sp. 3.5 x 10 ⁶ CFU/ml |
|--|-------------|---|---|
| 1.0% AMTicide [®] VAF | Day 7 | >99.999% | >99.999% |
| | Day 14 | >99.999% | >99.999% |
| | Day 21 | >99.999% | >99.999% |
| | Day 28 | >99.999% | >99.999% |
| 1.0% Phenoxyethanol | Day 7 | 68.785% | 88.963% |
| | Day 14 | 75.263% | >99.999% |
| | Day 21 | 88.123% | >99.999% |
| | Day 28 | >99.999% | >99.999% |
| Unpreserved Generic Cream | Day 7 | 2.631% | 2.879% |
| | Day 14 | 3.820% | 5.684% |
| | Day 21 | 4.631% | 6.196% |
| | Day 28 | 5.287% | 7.177% |

Figure 2. Headspace preservation test results. Chart shows percent (%) reduction of viable organisms.

Under the conditions of this test, the **AMTicide**[®] **VAF** exhibited the best preservation efficacy of headspace in a cosmetic and/or personal care product container compared to phenoxyethanol. The results of this study indicate **AMTicide**[®] **VAF** is capable of effectively protecting products exposed to an open environment of mold contamination.

REFERENCES

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3. Gu Y-Q, Mo M-H, Zhou J-P, Zou C-S, Zhang K-Q . 2007. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol. Biochem. 39:2567-2575.
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